CLATMS

- A method for preparing a cell extract for use in a cell-free protein synthesis means, comprising elimination of a cell-derived mechanism for inhibition of translation.
- The method according to claim 1, wherein the elimination of a cell-derived mechanism for inhibition of translation is provided by controlling ATP-mediated sugar phosphorylation pathway.
- 3. The method according to claim 1 or 2, wherein the cell-derived mechanism for inhibition of translation is an embryo cell-intrinsic inducible system of inhibition of protein synthesis.
- 4. The method according to any one of claims 1 to 3, wherein a source of the cell extract is a wheat embryo extract in which contaminating endosperm components and low molecular protein synthesis inhibitors are substantially removed.
- The method according to claim 1 or 2, wherein a source of the cell extract is E. coli, rabbit reticulocyte or insect cell extract.
- 6. The method according to claim 2, wherein the ATP-mediated sugar phosphorylation pathway is controlled by introducing at least one step selected from the followings:
 - 1) removing monosaccharides,
 - 2) removing phosphorylated sugars,

- controlling production of monosaccharides from polysaccharides, and
- controlling production of phosphorylated sugars from monosaccharides.
- 7. The method according to claim 6, wherein, in removing monosaccharides, the monosaccharide is a hexose.
- 8. The method according to claim 6, wherein the phosphorylated sugar is at least one selected from glucose 1-phosphate, fructose 1-phosphate, galactose 1-phosphate, glucose 1,6-diphosphate, fructose 1,6-diphosphate, galactose 1,6-diphosphate in removing phosphorylated sugars.
- 9. The method according to claim 6, wherein the monosaccharides and/or the phosphorylated sugars are removed by fractional elimination of molecular weight carried out by gelfiltration and/or with an ultrafiltration membrane.
- 10. The method according to claim 9, wherein the fractional elimination of molecular weight carried out by gel filtration and/or with an ultrafiltration membrane is repeated multiple times.
- 11. The method according to claim 6, wherein the production of monosaccharides from polysaccharides is controlled by controlling production of glucose from starch.
- 12. The method according to claim 11, wherein the

production of monosaccharides from polysaccharides is controlled by introducing at least one step selected from the followings:

- 1) removing or inactivating glycolytic enzymes,
- eliminating polysaccharides and/or oligosaccharides, and/or disaccharides, and
 - adding a glycolytic enzyme inhibitor.
- 13. The method according to claim 12, wherein a glycolytic enzyme is removed or inactivated by removing a complex between said glycolytic enzyme and calcium after forming the complex.
- 14. A method for preparing cell extract, wherein removal of a cell-derived glycolytic enzyme is introduced by adding at least one selected from bentonite, activated carbon, silica gel, Sephadex and sea sand to said cell extract as a precipitation auxiliary agent.
- 15. The method according to claim 6, wherein the production of phosphorylated sugars from monosaccharides is controlled by introducing at least one step selected from the followings:
- introducing an inhibitor against a sugar phosphorylation enzyme,
- $\begin{tabular}{ll} 2) removing or inactivating an sugar phosphorylation \\ enzyme, \end{tabular}$
- eliminating said production from glucose metabolic pathway by enzymatic degradation of a hexose,

- inhibiting an enzymatic reaction of sugar phosphorylation by chemical or enzymological modification of a hexose.
- 5) enzymatically and/or chemically alternating and/or modifying a phosphorylation site of the sugar, so that a phosphate group cannot bind to said phosphorylation site of the sugar.
- 16. The method according to claim 7, wherein the hexose is glucose.
- 17. The method according to claim 16, wherein a concentration of glucose in the cell extract is 10 mM or less when a concentration of the cell extract is 200 OD 260 nm.
- 18. The method according to claim 16, wherein a concentration of glucose in the cell extract is 6 mM or less when a concentration of the cell extract is 200 OD 260 nm.
- 19. The cell extract for use in a cell-free protein synthesis means prepared by the method according to any one of claims 1 to 18.
- 20. A cell extract for use in a cell-free protein synthesis means, wherein ATP-mediated sugar phosphorylation pathway is controlled.
- 21. The cell extract according to claim 20, wherein the ATP-mediated sugar phosphorylation pathway is controlled by introducing at least one step selected from the followings:

- substantially removing or inactivating phosphorylated sugars,
- substantially removing polysaccharides, oligosaccharides, disaccharides and monosaccharides,
- $\begin{tabular}{ll} 3) substantially removing or inactivating glycolytic \\ enzymes, \end{tabular}$
 - 4) adding a glycolytic enzyme inhibitor,
- substantially removing or inactivating phosphorylation enzymes,
 - 6) adding a phosphorylation enzyme inhibitor,
- 7) enzymatically and/or chemically alternating and/or modifying a phosphorylation site of the sugar, so that a phosphate group cannot bind to said phosphorylation site of the sugar.
- 22. A cell-free protein synthesis method using the cell extract according to any one of claims 19 to 21.
- 23. A use of cell-free protein synthesis system using the cell extract according to any one of claims 19 to 21.
- 24. A reagent kit for use in a cell-free protein synthesis system comprising the cell extract according to any one of claims 19 to 21.